

- c) determining from said intermediate statistical data a distribution of particles as a function of at least two arguments, wherein one argument is a specific brightness of the particles, or a measure thereof, and another argument is a diffusion coefficient of the particles, or a measure thereof,

wherein said distribution function of particles is determined by fitting the experimentally

determined probability functions $\hat{P}_1(\mathbf{n}_1), \hat{P}_2(\mathbf{n}_2), \dots$ by corresponding theoretical probability

functions $P_1(\mathbf{n}_1), P_2(\mathbf{n}_2), \dots$

and

wherein said theoretical distributions $P_1(\mathbf{n}_1), P_2(\mathbf{n}_2), \dots$ are calculated as functions of apparent concentrations and apparent brightness which depend on the width of the counting time interval.

19. The method according to claim 18, wherein the apparent concentration is calculated as

$$c_{app}(T) = \frac{c_{app}(0)}{\Gamma(T)},$$

the apparent brightness is calculated as

$$q_{app}(T) = q_{app}(0)\Gamma(T),$$

and $\Gamma(T)$ is calculated as

$$\Gamma(T) = \frac{1}{c_{app}(0)I_{app}(0)T^2} \int_0^T dt_1 \int_0^T dt_2 G(t_2 - t_1),$$

where $G(t)$ denotes autocorrelation function of fluorescence intensity.

20. The method according to claim 18 wherein, in calculations of the theoretical distributions

$P_1(\mathbf{n}_1), P_2(\mathbf{n}_2), \dots$, an optical spatial brightness function $B(\mathbf{r})$ is accounted for by a separately determined relationship between brightness B and volume elements dV .

21. The method according to claim 20, wherein the relationship between the spatial brightness

B and volume elements dV is expressed through a variable $x = \ln(B_0 / B)$ by a relationship

$$\frac{dV}{dx} = A_0(1 + a_1x + a_2x^2)x^{a_3}, \text{ where } B_0 \text{ is maximum brightness and } A_0, a_1, a_2 \text{ and } a_3 \text{ are}$$

empirical parameters of the spatial brightness function.

22. The method according to claim 18 wherein each set of counting time intervals consists of

intervals of equal width while different probability functions $\hat{P}_{T_1}(\mathbf{n}_1), \hat{P}_{T_2}(\mathbf{n}_2), \dots$ correspond

to counting time intervals of different widths T_1, T_2, \dots .

23. The method according claim 18 wherein the counting time intervals in each set are consecutive in time.

24. The method according to claim 18 wherein, counting time intervals in each set overlap.

25. The method according to claim 18 wherein said intermediate statistical data are processed applying inverse transformation with regularization and/or constraints.

26. The method according to claim 18 wherein the theoretical distributions $P_1(\mathbf{n}_1), P_2(\mathbf{n}_2), \dots$ are calculated through their generating functions $G_{P(\mathbf{n})}(\vec{\xi}) = \sum_{\mathbf{n}} \vec{\xi}^{\mathbf{n}} P(\mathbf{n})$.

27. The method according to claim 18 wherein said distribution function of particles is determined by fitting the experimentally determined probability functions $\hat{P}_1(n_1), \hat{P}_2(n_2), \dots$ by corresponding theoretical probability functions $P_1(n_1), P_2(n_2), \dots$.

28. The method according to claim 27, wherein the theoretical probability functions

$P_1(n_1), P_2(n_2), \dots$ are calculated through their generating functions $G_{P(n)}(\xi) = \sum_n \xi^n P(n)$.

29. The method according to claim 26 wherein the generating function is calculated using the expression where $c(q)$ is the density of particles with specific brightness q , T is the length of the counting time interval, and $B(\mathbf{r})$ is the spatial brightness profile as a function of coordinates.

30. The method according to claim 26 wherein said generating functions are calculated using the

formula $R_{P(n)}(\xi) = \exp[\sum_i c_i \int (e^{(\xi-1)q_i B(\mathbf{r})T} - 1) dV]$

in which c is an apparent concentration and q is an apparent brightness which both depend on the width of the counting time interval T .

31. The method according to claim 18 wherein concentrations of particles are selected to be approximately one or less molecules per measurement volume.

32. The method according to claim 18 wherein said photon detector is either an avalanche photodiode or a photomultiplier.

33. The method according to claim 18 wherein at least two photon detectors are used monitoring fluorescence of different wavelengths or polarization.
34. The method according to claim 18 wherein said fluorescent particles are characterized by applying an homogeneous fluorescence assay.
35. The method according to claims 18 for use in diagnostics, high throughput drug screening, optimization of properties of molecules and identification of specific cell populations.
36. Use of a confocal apparatus for performing the method according to claim 18.
37. Use of a confocal apparatus for performing the method according to claim 18, said confocal apparatus comprising:
- a) a radiation source (12) for providing excitation radiation (14),
 - b) an objective (22) for focusing the excitation radiation (14) into a measurement volume (26),
 - c) a detector (42) for detecting emission radiation (30) that stems from the measurement volume (26), and